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Polyalanine peptides doped with a small number of charged residues typically adopt alpha-helical conformations in aqueous solution if the number of residues exceeds a certain threshold value. Helical wheel projections of peptides based on the repeating unit $(AAKA)_n$ clearly illustrate the amphipathic nature of the peptides, with all the lysine residues residing on the same side of the helix. The amphipathic nature of these peptides distinguishes both them and their behavior in solution from alanine peptides of similar length and composition. At sub-millimolar concentrations, Ac-$(AAKA)_4$-$NH_2$ shows conformational instability over time upon dissolution in aqueous media. UV-Circular Dichroism (UV-CD) spectra indicate the presence of some α-helical structure at concentrations below ~ 100 μM, with increasing β-structural content as the concentration enters the millimolar regime. Above a certain threshold concentration (single digit millimolar), the peptide adopts a mostly β-like structure, which, upon salt addition, undergoes hydrogelation to form a network of fibers, as evidenced by Atomic Force Microscopy (AFM) images. Electronic and vibrational studies will be presented of both the gelled and un-gelled state, as will rheological and imaging studies of the hydrogel.

**463-Pos Board B342**

**Study of Misfolding and Aggregation for Short Peptide from the Yeast Prion Sup35 Using AFM Imaging and Force Spectroscopy**

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Protein misfolding is a fundamental pathway of protein self-assembly into nanoaggregates of various morphologies. However, our knowledge of this phenomenon is very limited. Protein aggregation is the cause of many conformational diseases justifying further study of protein misfolding phenomenon. We hypothesized earlier that the protein misfolded conformation is characterized by elevated intermolecular forces ultimately leading to aggregation. In this work we studied a short fragment (-GNNQQNY) of the yeast prion Sup35 which is critical for aggregation of the entire protein and presumably in the protein misfolding. By using force spectroscopy we measured forces for the interactions between a single pair of peptides. We established a correlation between aggregate morphology and strength of inter-peptide interactions as well as their pH dependence. The results of this study provide additional support for the importance of single molecule force spectroscopy for elucidating mechanisms of protein misfolding and aggregation. Using AFM imaging we also show that aggregates formed at different conditions (pH) for this short peptide exhibit distinctly different morphologies that cannot be predicted from the kinetics of aggregation study with ThT fluorescence. We found a dramatic difference of fibrils properties and their structure depending on the aggregation conditions. The difference in the aggregate properties was reflected in their adsorption to the surfaces having different properties: negatively or positively charged surface, PEG modified and hydrophobic surface. Salt concentration has also strong influence on kinetics and aggregate morphology with faster kinetics at higher salt concentration. We also show that the replacement of one amino acid residue in the sequence of this short peptide (Q4P) completely abolishes aggregation. Thus, the primary structure of the peptide is a critical determinant of aggregation propensity.

**464-Pos Board B343**

**On The Mechanisms Regulating Alpha-crystallin Activity**

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α-crystallin is a protein that plays several relevant physiological roles (i.e is the major constituent of human lens or help in maintain the correct folding of several protein) all of them affected by the occurrence of aggregation. α-crystallin supramolecular aggregation, induced by generating heat-modified α-crystallin forms, has been investigated over a range of temperature between 30°C and 60°C by means of static and dynamic light scattering and atomic force microscopy. Aggregation, after the formation of first clusters or basic aggregation units, can be described as a cluster-cluster aggregation similar to that of colloidal particles. Below a temperature $T_C = 45°C$, after a large lag time needed to form the first clusters, a fast, diffusion limited, aggregation can be observed. Above $T_C$ we observe a faster lag time followed by a slow aggregation. Correspondingly the temperature dependence of aggregation rates display an abrupt discontinuity at $T_C$ .
This discontinuity and the different kinetics of aggregation shed new light in the pathogenesis of the human eye lens cataract assigning a key role to the heat modified form of α-crystallin that markedly protect from aggregation preserving the transparency of the lens.

## Protein Aggregates II

**465-Pos Board B344**

**Effect Of Beta-sheet-breaker Peptides On The Assembly, Morphology And Mechanical Stability Of Oriented aß25-35 Amyloid Fibrils**

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Amyloid fibrils are self-associating filamentous structures that play an important role in neurodegenerative and protein misfolding diseases. It has been shown that certain peptides, called beta-sheet-breaker (BSB) peptides, may interfere with amyloid fibril assembly. Although BSB peptides are prospective therapeutic agents in amyloidosis, there is ambiguity about the mechanisms and generality of their action.
In the present work we analyzed the effect of LPFFD, Soto's BSB peptide, on the growth kinetics, morphological and mechanical properties of amyloid ß25-35 (Aß25-35) fibrils assembled in an oriented array on mica surface. Aß25-35 is thought to represent the biologically active, toxic fragment of the full-length beta peptide. Growth kinetics and morphological features were analyzed by using in situ AFM in the presence of various concentrations of LPFFD. The mechanical stability of the fibrils was explored with force spectroscopy methods. We found that the addition of LPFFD did not alter the assembly kinetics of Aß25-35 fibrils. Already formed fibrils did not disassemble in the presence of high concentrations of LPFFD. The nanomechanical behavior of Aß25-35 fibrils is characterized by the appearance of force plateaus which correspond to the force-driven unzipping of protofilaments. We observed that the plateau force did not change in the presence of LPFFD. The lack of significant effects of LPFFD on Aß25-35 fibril assembly and stability may suggest that the beta-sheet-breaking effect of the peptide is not general. Alternatively, the Aß25-35 fibrils formed on mica are in a configuration which is inaccessible to the LPFFD peptide.

**466-Pos Board B345**

**Thermal Stability Of Oriented Aß25-35 Amyloid Fibril Nanoarray**

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Amyloid fibrils are filamentous protein deposits in the extracellular space of various tissues in neurodegenerative and protein misfolding diseases. It has been proposed that amyloid fibrils may be used in nanotechnology applications because of their self-assembly properties and stability. Recently we have shown that amyloid beta 25-35 (Aß25-35) forms a highly oriented, potassium-dependent network on mica. The mutant form of the peptide (Aß25-35_N27C), which forms an identically oriented nanoscale network, may be chemically addressed for functionalization in dedicated applications. In order to utilize the amyloid nanoarray in nanotechnology applications, uderstanding its physical and chemical stability is important.
In the present work we investigated thermally induced changes in the morphology of the oriented Aß25-35 fibril network. The fibrils maintained high orientation stability in the temperature range of 30-70 degrees, suggesting that orientational rearrangement of Aß25-35 fibrils on mica is an unfavorable process. Above 45 degree a gradual decrease in fibril length and dissociation from the surface could be observed. In addition, at high temperatures (45-70 degrees) the average fibril thickness increased, indicating changes in the underlying structure or structural dynamics. Possibly, a thermally induced transition in the Aß25-35 peptide around 45 degree leads to structural changes in the fibril as well. The temperature-dependent changes described here need to be considered in the use of amyloid fibrils in nanotechnology applications.

**467-Pos Board B346**

**Fibril formation of Aβ (10-35) studied by UV resonance Raman Spectroscopy**

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In Alzheimer's disease the major pathological feature observed is the progressive deposition of insoluble senile amyloid plaques within the cerebral cortex. The major component of these plaques is amyloid beta (Aβ), a 39-43 residue

peptide. This peptide can be present as monomers with soluble disordered structure, which change conformation into partially folded intermediates and further assemble to form dimers, trimers, oligomers, protofibrils and larger fibrils. Earlier studies have suggested that neurotoxicity of the disease lies not in the insoluble fibrils but in the formation of soluble oligomers, which impair and alter neurotransmision. Thus, the solution conformation of Aβ is of significant interest for understanding the molecular mechanism of Aβ fibrillogenesis. In the present study we have investigated the process of fibril formation in the 25 residue Aβ(10-35) which has a similar fibril structure as $A\beta_{40}$ and $A\beta_{42}$. In addition, Aβ(10-35) contains the central hydrophobic cluster (residues 17-21) suspected to initiate folding.

We have employed fluorescence, circular dichroism (CD) and time-dependent UVRR spectroscopic methods to explore the fibril formation process in vitro. Thioflavin-T induced fluorescence and CD studies indicate a conformational transition from an unfolded to β-sheet structure. UVRR studies conducted with 215 nm and 230 nm excitation facilitate characterization of the two phenylalanine and one tyrosine residues in the peptide, respectively. It is observed that formation of soluble Aβ oligomers is accompanied by a decrease in the intensities of the Phe vibrational mode, v8a (1606 $cm^{-1}$) and the Tyr vibrational mode, v8a (1617 $cm^{-1}$) over a 150 min incubation period. These results indicate that fibril formation proceeds via phenylalanine and tyrosine π-stacking interactions, which stabilize parallel β-sheets and reduce solvent exposure. Based on these results we have proposed a probable mechanism of fibril formation in Aβ(10-35).

**468-Pos Board B347**

**Amyloid Beta Oligomer Formation Analysis by Photon Counting Histogram**

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Amyloid beta (Ab) is 4 kDa peptide which is thought to form aggregates such as oligomers and fibrils, and to cause Alzheimer disease (AD). Recently, it has been suggested that soluble Ab oligomers are the causative agent of AD since such oligomers are more cytotoxic than fibrils. It was also suggested that Ab oligomers affect not only cell death but also early stage of cell dysfunction and cause memory loss.

However, the mechanism how soluble oligomers are produced is still unknown. In this study, we analyzed formation of Ab oligomers in vitro at a single molecule level using photon counting histogram (PCH)(Chen, Y. et al. (1999) *Biophys. J.* 77, 553-67; Terada, N. et al. (2007) *Biophys. J.* 92, 2162-71.). Using PCH method, the number of protomers in oligomers and concentrations are obtained from histograms of photons from fluorescent molecules. Combination of a conforcal optics and a photon counting sensor enables us to catch the fluorescence from molecules diffusing through the conforcal volume at PCH. The concentration distribution of oligomers can be calculated from histograms.

Fluorescent intensity of fluorescein-labeled Ab monomer (FL-Ab) was evaluated using PCH. After 30 min incubation of FL-Ab in buffer solution, dimer fraction was successfully observed with PCH, assuming that fluorescent intensity is in proportion to the number of protomers in oligomers. Detailed analysis of formation of Ab oligomers such as amyloid-beta derived diffusible ligands (ADDLs) using these techniques is in progress.

**469-Pos Board B348**

**The Aggregation of Aβ 16-22 Probed by Circular Dichroism and Infrared Spectroscopies**

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The misfolding of certain proteins into β-sheets and their ensuing aggregation into fibrous networks that accumulate to form insoluble plaques has been found to be related to several neurodegenerative disorders such as Alzheimer's Disease, Huntington's Disease, and the spongiform encephalopathies. The structural details of these protein aggregates and their mechanisms of aggregation, however, have not yet been fully understood. This research analyzes the structure and misfolding processes of a fragment of the β-amyloid (Aβ) polypeptide comprising residues 16 - 22 (KLVFFAE). Aβ 16-22 aggregates by forming intermolecular antiparallel β-sheets; lamination of these sheets results in formation of fibrils or micro-crystallite structures. We have probed the conformation of Aβ 16-22 as a function of concentration, temperature, and solvent using circular dichroism (CD) and infrared (IR) spectroscopies. The data show that, as the concentration increases, the conformation of Aβ 16-22 changes from a random coil to a β-sheet to higher-order structures; these higher order structures have a unique CD signature, with enhancement of the positive feature at ~200 nm. Addition of hexafluoroisopropanol (HFIP) disrupts the β-sheet structure, and the kinetics of this change can be observed spectroscopically. Overall, these observations allow us to construct a model of the mechanism of Aβ 16-22 aggregation.

**470-Pos Board B349**

**Conformational Change Induced In A Random Coil Peptide By Prion Peptide Aggregates**

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The *in vivo* aggregation of many different polypeptides into β-sheet-rich amyloid fibers is associated with a range of diseases. The mechanism of aggregation for many of these proteins is still unknown; small polypeptides are useful models for exploring this process by both physical and computational methods. H1, a peptide derived from residues 109-122 of the Syrian hamster prion peptide (Ac-MKHMAGAAAAGAVV-$NH_2$) forms antiparallel β-sheet aggregates in solution. As determined by isotope-edited infrared (IR) spectroscopy, the H1 β-sheets have three overhanging residues at the N-terminus, with residue 117 aligned in all strands (1). The A117G mutant, however, exists nearly entirely as monomer as a result of backbone flexibility and the inability to align across residue 117 (2). In this study, the results of mixing wild-type H1 and A117G were monitored by Fourier Transform infrared spectroscopy (FTIR). Regardless of their relative concentrations, combining H1 and A117G resulted in the spontaneous, irreversible conversion of A117G from its unordered form to β-sheet by a mechanism in which the H1 peptides are proposed to serve as a nucleating template. Isotope-edited IR studies suggest that the β-sheet formed after mixing H1 and A117G is composed of alternating strands from each peptide variant. This process occurs spontaneously over time, and its rate is accelerated with increasing temperature. This work suggests a model for the templating of an unordered polypeptide into a β-sheet fibril.

(1) Silva, R.A.G.D., W. Barber-Armstrong and Decatur, S.M. (2003) *J. Am. Chem. Soc.* **125**: 13674.
(2) Petty, S.A., Adalsteinsson, T., and Decatur, S.M. (2005) *Biochemistry* **44:** 4720.

**471-Pos Board B350**

**Successful de novo conversion from [psi−] to [PSI+] Saccharomyces cerevisiae**

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Prion protein disorders, such as transmissible spongiform encephalopathies (TSEs) and Creutzfeldt-Jacob Disease (CJD), are caused by amyloid aggregation within a cell. The amyloid aggregates are proteinaceous, rich in β-pleated sheets, and characterized by protease resistance. *Saccharomyces cerevisiae* naturally expresses Sup35, a protein that spontaneously aggregates and forms intracellular amyloid fibers. Sup35 exhibits multiple prion states: [*psi*$^-$], strong [*PSI*$^+$], and weak [*PSI*$^+$] phenotypes. Prior studies have induced multiple [*PSI*$^+$] phenotypes by using *in vitro* aggregation reactions of bacterially expressed Sup35NM at varying temperatures and then in some instances transforming *S. cerevisiae* with these aggregate ''seeds.'' By implementing a unique color assay, the different phenotypes may be visually identified as red, pink, and white colonies for [*psi*$^-$], weak [*PSI*$^+$], and strong [*PSI*$^+$], respectively. Here our research focused on inducing strong [*PSI*$^+$] and weak [*PSI*$^+$] prion states from [*psi*$^-$] cells without the use of preformed prion seeds. We have found that exposure of mid-exponential phase cultures of [*psi*$^-$] 74-D694 yeast to a 4°C environment for an extended period of time induced *de novo* an array of [*PSI*$^+$] phenotypes. Our current focus is on purifying the amyloids of these phenotypic variants and analyzing their secondary structure and conformations using a variety of techniques, including transmission electron microscopy and X-ray diffraction. We will also compare the structural data from the *de novo* amyloid with those of amyloid fibers that formed *in vitro* from bacterially-expressed Sup35NM.

**472-Pos Board B351**

**Conversion Of Antiparallel β-sheet To Parallel β-sheet In A Prion Peptide Aggregate**

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Residues 109-122 of the Syrian hamster prion peptide (Ac-MKHMAGAA AAGAVV-$NH_2$) make up a conserved amyloidogenic portion of a protein associated with several neurodegenerative diseases. In previous studies using Fourier Transform infrared spectroscopy (FTIR) and isotope-edited infrared spectroscopy, H1 has been determined to form antiparallel β-sheets in solution with three overhanging residues at the N-terminus and alignment of residues 117 in all strands (1, 2). Some amyloidogenic proteins form antiparallel β-sheet